STIC-ILL

Vol 10

Claims 4/28/91

only

From: Holleran, Anne
Sent: Friday, January 17, 2003 11:58 AM
To: STIC-ILL
Subject: references for 09/786,502

Please send copies of the following papers:

1.	Gong	Cancer and Metastasis Reviews (2000) Volume date 1999,18(4): 483-490
2.	Liao	Biotechnology and Bioengineering (2001) 73(4): 313-323
3.	Cohen	Methods in Molecular Biology (2002) 178: 367-378
4.	Paola	J. Molecular Biology (2002) 320(1): 1-10
5.	Bach	J. Molecular Biology (2001) 312(1): 79-93
6.	Cohen	Oncogene (1998) 17(19): 2455-1456
7.	Darcy	European J. Immunology (1998) 28(5): 1663-1672
8.	Proba	J. Molcular Biology (1998) 275(2): 245-253
9.	Mhashilkar	Intrabodies (1998) 147-161. Editor, Marasco, Wayne A.; Springer: Berlin, Germany
10	Fitzer-Attas	J. Immunology (1998) 160(1): 145-154
11.	Curiel	Intrabodies (1998) 97-128. Editor, Marasco, Wayne A.; Springer: Berlin, Germany
12.	Hassanzadeh	FEBS Letters (1998) 437(1-2): 81-86
13.	Hassanzadeh	FEBS Letters (1998) 437(1-2) 75-80
14.	Beerli	Intrabodies (1998) 61-74. Editor, Marasco, Wayne A.; Springer: Berlin, Germany
15.	Mhashilkar	EMBO Journal (1995) 14(7): 1542-1551
16.	Chen	Human Gene Therapy (1994) 5(5): 595-601

Anne Holleran
AU: 1642
Tel: 308-8892
RM: 8e03

mailbox: 8e12

9380268

Cancer and Metastasis Reviews **18:** 483–490, 1999.

Prostate-specific membrane antigen (PSMA)-specific monoclonal antibodies in the treatment of prostate and other cancers

Michael C. Gong[1,2], Sam S. Chang[1], Michel Sadelain[2], Neil H. Bander[3] and Warren D.W. Heston[1]
[1]*Department of Urology,* [2]*Department of Human Genetics, Memorial Sloan-Kettering Cancer Center, New York;* [3]*Department of Urology, New York Presbyterian Hospital-Weill Medical College of Cornell University, New York, NY, USA*

Key words: monoclonal antibody, immunotherapy, angiogenesis, gene therapy

Abstract

Prostate-specific membrane antigen (PSMA) is a cell surface glycoprotein that is expressed by prostate epithelial cells. PSMA-specific monoclonal antibodies have been utilized to characterize the biologic function and *in vivo* biodistribution of PSMA. PSMA is an attractive target protein for monoclonal antibody directed imaging or therapeutics for prostate cancer since its expression is relatively restricted to prostate epithelial cells and is over-expressed in prostate cancer, including in advanced stages. Currently, clinical usage of PSMA specific monoclonal antibodies has been limited to diagnostic immunohistochemistry and imaging of patients with prostate cancer. Novel applications for these antibodies will be discussed.

Introduction

Monoclonal antibodies (mAbs) have been an invaluable tool in basic science research for over 20 years. In the clinical setting, mAbs are primarily used for either detection of antigens or to specifically target therapeutic molecules to cells expressing a particular antigen. In oncology, mAbs have been utilized for diagnosis (immunohistochemistry), clinical monitoring (serum assays), clinical staging (radiolabeled imaging) and tumor targeting (immunotherapy). Membrane proteins are ideal targets for mAb directed imaging or therapeutics. Ideally, the most specific mAbs are those that recognize an epitope within the extracellular domain of the membrane protein. This review will focus on the application of prostate-specific membrane antigen (PSMA)-specific mAbs for the diagnosis and treatment of prostate cancer and possibly other solid tumors.

PSMA: gene, structure and function

Using the 7E11 mAb [1], the gene encoding PSMA was cloned at Memorial Sloan-Kettering from the human prostate cancer cell line LNCaP and PSMA was found to be a 100 kDa type II membrane glycoprotein [2]. LNCaP cells abundantly express PSMA, whereas other human prostate cancer cell lines, PC-3 and DU-145, do not express PSMA [3]. The gene encoding PSMA has been localized to chromosome 11p11–p12 and a homologous PSMA gene is located on chromosome 11q14 [4–6]. PSMA was subsequently discovered to have two enzymatic activities, one is a folate hydrolase [7] and the other is a carboxypeptidase [8, 9]. PSMA is an exopeptidase capable of hydrolyzing γ-glutamyl linkages in polyglutamated folate and methotrexate triglutamate [7]. Thus, the nomenclature committee of the International Union of Biochemistry and Molecular Biology has recommended for PSMA the formal name of glutamate carboxypeptidase (EC 3.4.17.21 [10]). However, the function of PSMA in the prostate and its role in prostate cancer remain to be elucidated.

PSMA-specific monoclonal antibodies

The first and most widely characterized mAb specific for PSMA was described in 1987, named 7E11 [1].

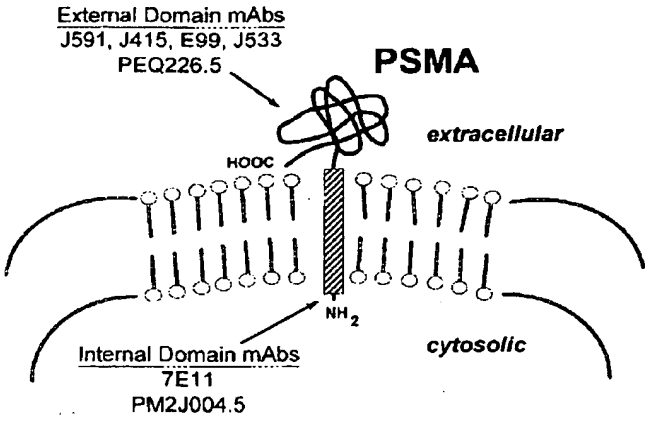

Figure 1. PSMA-specific mAb epitope localization. MAb epitope cellular location of PSMA-specific mAbs used by Chang et al. [22].

The 7E11 epitope has been mapped to the first 6 amino acids of the amino terminus of PSMA [11]. Because the 7E11 mAb recognizes an epitope that is located within the cell membrane, 7E11 does not bind viable cells expressing PSMA [12]. As a result, several investigators have developed mAbs that recognize epitopes within the extracellular domain of PSMA and thus bind viable cells expressing PSMA [12–14]. Liu et al. described four mAbs, named J591, J533, J415 and E99, that recognize different epitopes of the extracellular domain of PSMA [12]. Two mAbs have also been developed by the researchers at Hybritech, PEQ226.5 (extracellular epitope) and PM2J004.5 (intracellular epitope) [13]. Figure 1 summarizes the cellular domain recognized by the above PSMA-specific mAbs. Recently, another 5 mAbs with specificity to the extracellular domain, named 3E11, 3C2, 4E10-1.14, 3C9 and 1G3, have been reported [14]. Together, these mAbs have been utilized in multiple investigations to characterize the function and biodistribution of PSMA.

PSMA localization

PSMA is expressed by the secretory acinar epithelium in normal prostate. Furthermore, using mAb 7E11, PSMA expression has been described as increased in prostatic intraepithelial neoplasia (PIN) and adenocarcinoma of the prostate [1, 3, 15–17]. In a recent study, 184 radical prostatectomy specimens were examined for PSMA expression using 7E11. The percentage of cells expressing PSMA was reported to be incrementally increased from benign prostate epithelium (69%) to high grade PIN (78%) to prostate cancer epithelium (80%). In addition, the highest grade tumors displayed the highest intensity staining [16]. In a subsequent study, specimens from 232 patients with lymph node metastasis who underwent radical prostatectomy and pelvic lymph node dissection were examined for PSMA expression using 7E11. The percentage of cells expressing PSMA was reported as 46% of benign prostate epithelium, 79% of prostate cancer epithelium and 76% of lymph node metastasis. Interestingly, the intensity of PSMA staining was the highest in the prostate cancer epithelium but the lowest in the lymph node metastasis [17].

In contrast to the widely utilized prostate cancer serum marker, prostate-specific antigen (PSA), PSMA expression appears to be inversely related to androgen levels. PSMA expression was increased when LNCaP cells were incubated in the presence of androgen deprived media whereas LNCaP cells incubated in the presence of the androgen dihydroxytestosterone had decreased PSMA expression [3]. Similarly, in prostate cancer specimens from patients treated with either castration or long-term androgen deprivation, PSMA expression was increased in the prostate in 11/20 patients and in prostate cancer metastasis from 4/4 patients. In contrast, PSA expression was found to be decreased in the prostate in 14/20 patients and in prostate cancer metastasis from 4/4 patients [18]. *In situ* hybridization studies revealed increased PSMA expression in prostate cancer biopsies from patients with hormone refractory disease as well as patients with prostate cancer with increased Gleason's score [19]. Thus, manipulation of the androgen levels in patients with prostate cancer may affect PSMA expression.

In this light, PSMA expression in 20 radical prostatectomy specimens from patients who were treated with 3 months of neoadjuvant androgen deprivation were compared to PSMA expression in a similar cohort of 20 specimens from patients who were treated with radical prostatectomy alone. There was no significant increase in PSMA expression in the androgen deprivation group [20]. One possible explanation for this discrepancy is that the 3 month androgen deprivation time course was too short and subtle changes in PSMA expression may be too difficult to delineate at the immunohistochemical level. Another possible explanation is that the specimens were obtained from patients with predominantly well-differentiated tumors rather than patients

with advanced or hormone-refractory prostate cancer. Further investigations with more sensitive techniques to detect PSMA expression such as *in situ* hybridization may be necessary to determine if androgen deprivation can be used to alter *in vivo* PSMA expression.

Similar to other tumor-associated antigens, PSMA is not exclusively expressed in prostatic tissues. Several other normal non-prostatic tissue types have been shown to express PSMA, albeit expression is less intense and in some tissues is inconsistent. PSMA expression in non-prostatic tissue was originally described in 1987 using the mAb 7E11 in immunohistochemical studies, demonstrating expression in the kidney but not small intestine, brain, skeletal muscle, colon, heart or breast [1]. When utilizing ribonuclease protection assays PSMA expression was found in small intestine, brain and salivary glands, but not in muscle, kidney, liver or mammary glands [3]. Western blot analysis using 7E11 demonstrated expression in small intestine, brain and salivary glands but not kidney, skeletal muscle, colon, heart, liver or breast tissues [21].

In order to better define the biodistribution of PSMA and reduce inconsistencies observed in the PSMA localization in non-prostatic tissue, a recent comprehensive immunohistochemical investigation using mAbs: 7E11, J591, J415, PEQ226.5 and PM2J004.5 was performed. Chang et al. convincingly demonstrated that PSMA localizes in the duodenal columnar (brush border) epithelium, proximal renal tubular epithelium and breast epithelium but not the brain, liver, colonic epithelium, lung, bladder, testis or several other tissues (Table 1). Interestingly, only 7E11 reacted with skeletal muscle whereas the other four PSMA mAbs did not [22]. Furthermore, *in situ* histochemical studies confirmed the immunohistochemical localizations [23].

The proximal small bowel is a tissue with high folate hydrolase activity and thus the PSMA mAb cross-reactivity may coincide with the intestinal enzymatic activity for absorbing folates from ingested food sources. The proximal tubule cells of the kidney also have been shown to have a role in folate reabsorption at the apical membrane, which may also explain the cross-reactivity in the kidney. The skeletal muscle staining seen with the 7E11 mAb but not the others mAbs may represent non-specific binding of 7E11 to this tissue.

Interestingly, PSMA is also expressed in the neovasculature of all solid tumors that have been investigated [12, 22–24]. Initially, 7E11 was used to demonstrate immunohistochemical staining of the endothelium of neovasculature of renal cell carcinoma, transitional cell carcinoma of the bladder and colon carcinoma [24]. Using mAbs with specificity for the extracellular domain, Liu et al. demonstrated staining of the tumor-associated neovasculature in 23/23 non-prostatic carcinomas that included renal, urothelial and lung carcinomas [12]. Chang et al. utilized the mAbs: 7E11, J591, J415, PEQ226.5 and PM2J004.5 to examine the expression of PSMA in the neovasculature of a wide number of carcinomas (Table 1). Tumor-associated neovascular endothelial cells were identified by immunohistochemical staining of CD34 in sequential tissue sections. CD34 is widely used as an endothelial marker for angiogenesis and microvessel density determination. PSMA co-localized with the CD34 expressed in tumor-associated neovasculature. Vessels in adjacent non-cancerous tissue did not display immunoreactivity and the vasculature of the corresponding benign tissue also did not demonstrate PSMA expression. Additionally, the malignant cells themselves did not display immunoreactivity [22]. The PSMA immunohistochemical studies localizing the antigen to the tumor-associated neovasculature were later confirmed by RT-PCR and *in situ* histochemistry, which demonstrated localization of PSMA transcripts [23].

The neovasculature expression of PSMA in solid tumors, however, did not seem to occur in prostate carcinoma [16, 17, 22]. The reason for lack of expression in the neovasculature of prostate carcinoma remains unclear. Carcinomas of the prostate do not classically demonstrate typical angiogenic characteristics compared to many of the other solid tumors. In addition, prostate carcinomas lack a significant stromal desmoplastic response and thus this may inhibit PSMA expression in the neovasculature. Other inhibitory factors or the presence of PSMA in the carcinoma itself may be limiting the neovascular expression in prostate carcinomas.

Diagnostic clinical applications

Currently, the only clinical application of PSMA-specific mAbs is in clinical staging. The mAb 7E11 has been modified by the linkage of ^{111}Indium, creating a radiolabeled diagnostic marker for *in vivo* detection of prostate cancer recurrence and metastasis. This diagnostic test marketed under the name ProstaScint

Table 1. PSMA-specific mAb immunohistochemical reactivity

Tissue type	Immunoreactivity	Reactivity site
Benign		
Prostate	Y	Acinar ducts
Lung	n	
Brain	n	
Parotid	n	
Esophagus	n	
Stomach	n	
Duodenum	Y	Brush border, columnar epithelium
Ileum	n	
Pancreas	n	
Liver	n	
Kidney	Y	Proximal tubules
Bladder	n	
Testis	n	
Breast	Y	Epithelial tissue
Ovary	n	
Skin	n	
Thyroid	n	
Adrenal cortex/medulla	n	
Neoplasm		
Conventional renal cell carcinoma	Y	Neovasculature
Transitional cell carcinoma	Y	Neovasculature
Testicular embryonal carcinoma	Y	Neovasculature
Colonic adenocarcinoma	Y	Neovasculature
Neuroendocrine carcinoma	Y	Neovasculature
Glioblastoma multiforme	Y	Neovasculature
Malignant melanoma	Y	Neovasculature
Pancreatic duct carcinoma	Y	Neovasculature
Non-small cell lung carcinoma	Y	Neovasculature
Soft tissue sarcoma	Y	Neovasculature
Breast carcinoma	Y	Neovasculature
Hemangioma	n	
Hemangioendothelioma	n	
Angiosarcoma	n	
Angiolipoma	n	
Angiomyolipoma	n	
Prostatic adenocarcinoma	Y	Malignant cells, sporadic neovasculature

Tissues were stained with PSMA-specific mAbs 7E11, J591, J415, PEQ226.5 and PMJ004.5 [22]. Immunoreactivity between the mAbs was consistent in the above tissue type.

(Cytogen, Princeton, NJ) gained FDA approval for clinical usage in 1997. Clinical trials have demonstrated both few adverse side effects and the clinical utility of radiolabeled mAb 7E11 in the identification of prostate cancer recurrence and metastasis [25–27]. In an early study, 27 patients with biochemical evidence of prostate cancer recurrence evidenced by a rising PSA were imaged with the ProstaScint scan; 22/27 patients had a demonstrable lesion on the scan and 11 of the 22 had confirmation by other means [28]. In a follow-up study, 183 patients with similar stage of disease were scanned and similarly, 50% of the patients with a positive scan were confirmed to have recurrence by biopsy [27]. The ProstaScint scan was found to be superior to CT and positron emission tomography (PET) scans in the detection of prostate cancer recurrence and metastasis. Overall, the ProstaScint scan demonstrated a 60–80% sensitivity rate and a 70–90% specificity rate for detecting prostate cancer recurrence or metastasis [25–27].

One potential limitation of the ProstaScint scan is that the 7E11 mAb recognizes an epitope which is

intracellular and thus theoretically, the radiolabeled antibody may only be recognizing dying or apoptotic prostate cancer cells. An attractive alternative to increase sensitivity may be to use a radiolabeled mAb that recognizes an extracellular epitope of PSMA. Currently, radiolabeled mAb J591 is under investigation as a diagnostic tool. Finally, an incidental renal cell carcinoma was recently discovered by the ProstaScint scan [29], indicating that localizing PSMA expression in tumor-associated neovasculature may be another *in vivo* antigen targeting application for PSMA mAbs.

Detection of circulating cancer cells has been implicated in poor prognosis in staging patients with cancer. The most common assay used is reverse-transcribed mRNA detection using the polymerase chain reaction, commonly abbreviated RT-PCR. Circulating prostate cancer cells are detected by utilizing the RT-PCR technique to detect cells expressing either PSA or PSMA [30, 31]. To date, studies using the RT-PCR assays to predict prognosis have been inconsistent. One of the reasons that the data has been inconclusive is that the RT-PCR technique is difficult to reliably reproduce from laboratory to laboratory. One factor that contributes to the variable results with PSMA-based RT-PCR is contamination of the reaction with genomic DNA or possibly transcripts from the PSMA-like gene on chromosome 11q14. One possible alternative to detect circulating prostate cancer cells is to use PSMA-specific mAbs, however, the primary current limitation is level of sensitivity. Another alternative strategy to increase sensitivity and specificity is to first capture circulating prostate cancer cells with a mAb with a relatively broad specificity, such as an epithelial antigen, and subsequently perform PSMA-specific RT-PCR on the isolated cells [32]. Further studies to improve sensitivity, specificity and reproducibility are under investigation.

Monoclonal antibody directed immunotherapy

Immunotherapy strategies utilized to treat cancer are categorized as either active or passive. Active immunotherapy strategies attempt to elicit an anti-cancer immune response within the patient. Passive (or adoptive) immunotherapy strategies utilize the infusion of pre-formed anti-cancer effector molecules to treat patients with cancer. These effector molecules are typically proteins, such as antibodies or whole cells that have a direct immune-based anti-cancer activity. Traditionally, mAbs have been used in adoptive immunotherapy trials to target tumor antigen expressing cancer cells by infusing mAbs themselves or mAbs that have been conjugated to toxins or radioisotopes. MAbs that bind the extracellular domain of PSMA have been shown to bind viable cells and become internalized [33]. By being internalized, these mAbs may be more attractive for directing PSMA-specific delivery of tumor toxins, radioactive particles or drugs. Clinical usage of PSMA-specific mAbs conjugated to toxins and radioactive particles is under current investigation.

More recently, strategies have developed using mAbs to attempt to target immune cells to the tumor. Artificial T cell receptors that incorporate the antigen-specific moiety of an immunoglobulin provide a means to target antigens independently of major histocompatibility complex (MHC) expression. One advantage is that T cells expressing this type of artificial receptor will recognize *native* tumor-associated antigens on the tumor cell surface. Another advantage is that one mechanism tumors may evade the immune system is through loss of MHC expression such that cytotoxic T cells will no longer recognize tumor cells. The artificial T cell receptor does not require tumor cell MHC expression and thus would confer tumor antigen specificity to T cells, independent of MHC expression. A third advantage is that because the artificial receptor is not MHC restricted, the same receptor may be used in any individual.

For the purposes of genetic engineering, single genes are more readily transferred into cells. However, mAbs consist of two proteins, a heavy chain and a light chain that are linked through a disulfide bond. Since two separate genes encode the heavy and light chains, both genes must be expressed by a cell to synthesize the mAb. A single chain antibody (scFv) is an antibody that fuses the domains of the heavy and light chain that are responsible for antigen recognition into one single continuous gene. A scFv-based ζ fusion artificial T cell receptor (Figure 2) can be encoded by one gene and utilized in gene therapy strategies (for review see [34]).

A PSMA-specific single chain antibody was cloned from the J591 hybridoma, which produces a mAb that recognizes the extracellular domain of PSMA (Gong MC et al., unpublished data). The single chain antibody was then cloned to the CD8 hinge and transmembrane domain followed by the cytoplasmic domain of the CD3-associated ζ chain, creating a PSMA-specific artificial T cell receptor named Pz-1 [35]. By taking advantage of efficient retroviral-mediated gene transfer, the Pz-1 receptor was utilized to reprogram primary

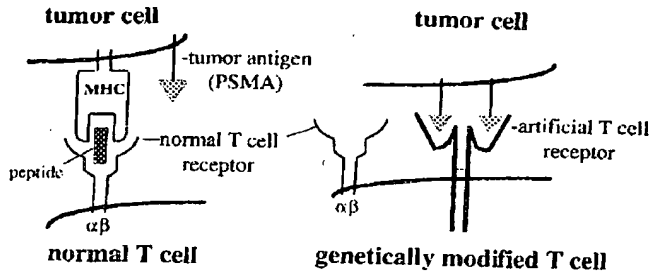

Figure 2. Schematic diagram of scFv-based ζ fusion artificial T cell receptor. Left diagram: Normal cytotoxic T cell recognition of tumor cell. Specific T cell recognition and cytolysis of tumor cell requires specific interaction between the T cell receptor (consisting of an α and β chain) with tumor-specific peptide in context with MHC class I molecule. Right diagram: T cell genetically modified with scFv-based fusion artificial T cell receptor. The artificial T cell receptor re-programs the T cell, altering the T cell specificity, to recognize tumor-associated antigens in their native state, independent of MHC.

human T cells. In cytotoxicity assays, the Pz-1 modified T cells were capable of specifically recognizing and lysing prostate cancer cells expressing PSMA (Figure 3)

Additionally, the Pz-1 modified T cells were able to proliferate and release both interleukin-2 and interferon-γ in response to cells expressing cell surface PSMA. Furthermore, these cellular responses of proliferation and cytokine secretion were augmented by B7.1-mediated co-stimulation [35]. Finally, because T cell signaling defects have been reported in patients with cancer, the Pz-1 receptor was transduced into T cells from 5 patients with prostate cancer. The Pz-1-modified T cells from all 5 patients with prostate cancer also were capable of lysing prostate cancer cells expressing PSMA, independent of the clinical stage or age of the patient [35]. These results together demonstrated that the PSMA-specific artificial T cell receptor, Pz-1 is capable of efficiently reprogramming T cells from prostate cancer patients to recognize PSMA in a MHC independent fashion *in vitro*. Current investigations are underway to examine the efficacy and safety of the Pz-1-receptor-modified T cells in animal models.

Clinical applications using PSMA-specific mAbs may not be limited to the treatment of prostate cancer. Angiogenic targeting is a new intriguing and exciting cancer therapy under intense investigation. All malignancies require angiogenesis to thrive and it is this neovasculature that expresses PSMA. Because there is a clear difference in PSMA expression in vessels associated with tumors in contrast to its absence in vessels of normal tissue, PSMA is a potential unique anti-angiogenic target. Current endothelial target antigens such as vascular epidermal growth factor (VEGF) receptors, CD31 and α-v-β-3 integrins are upregulated in malignancies but are also present in normal vessels. Thus, targeting these proteins may be toxic to normal vasculature. By targeting the PSMA expressed in the tumor-associated neovasculature, other non-prostatic malignancies may be targets of PSMA-specific therapies. The potential applications of PSMA-specific therapies must also consider the fact that these antibodies also can localize to other non-prostatic tissues. Although localization to these other non-prostatic tissues has not been a significant problem in the usage of the ProstaScint scan, further investigations are necessary to determine the side effects of PSMA-directed therapies.

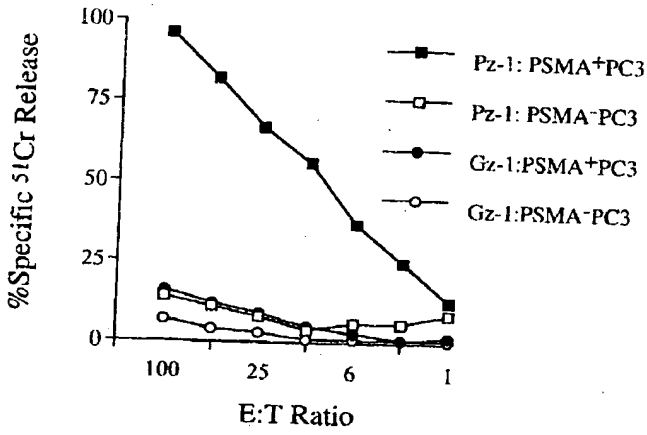

Figure 3. Pz-1-modified T cells display PSMA-specific cytotoxicity. Using efficient retroviral-mediated gene transfer, primary human T cells were transduced with the Pz-1 receptor. Transduced T cells were incubated with ^{51}Cr-labeled target cells at various effector to target (E : T) ratios for 4 h. Target cells were the human prostate cancer cell line PC3, which normally do not express PSMA (PSMA$^-$PC3) or PC3 cells genetically engineered to express PSMA (PSMA$^+$PC3) [35]. % Specific ^{51}Cr release was calculated by (^{51}Cr release − spontaneous release)/(maximum ^{51}Cr release − spontaneous release) × 100. Experiments were performed in triplicate. Pz-1-modified T cells specifically recognized PSMA$^+$PC3 cells (■) but not native, PSMA$^-$PC3 cells (□). T cells modified with an irrelevant scFv-based artificial T cell receptor, Gz-1, do not recognize either native PSMA$^-$PC3 cells (○) or PSMA$^+$PC3 cells (●).

Conclusions

PSMA is a tumor-associated antigen that is overexpressed in prostate cancer whose expression is further increased in advanced stages. Thus, PSMA is an excellent prostate cancer target for both diagnostic and therapeutic modalities. Investigations on the usage of PSMA-specific mAbs continue to be developed. Ideally these mAbs and their derivatives will display a prolonged *in vivo* half-life but still not elicit a significant host immune response against the mAb itself. Finally, PSMA may be a novel target for future antiangiogenic therapy in the treatment of other malignant solid tumors.

References

1. Horoszewicz JS, Kawinski E, Murphy GP: Monoclonal antibodies to a new antigenic marker in epithelial prostatic cells and serum of prostatic cancer patients. Anticancer Res 7: 927–35, 1987
2. Israeli RS, Powell CT, Fair WR, Heston WD: Molecular cloning of a complementary DNA encoding a prostate-specific membrane antigen. Cancer Res 53: 227–30, 1993
3. Israeli RS, Powell CT, Corr JG, Fair WR, Heston WD: Expression of the prostate-specific membrane antigen. Cancer Res 54: 1807–11,1994
4. Rinker-Schaeffer CW, Hawkins AL, Su SL, Israeli RS, Griffin CA, Isaacs JT, Heston WD: Localization and physical mapping of the prostate-specific membrane antigen (PSM) gene to human chromosome 11. Genomics 30: 105–8, 1995
5. Leek J, Lench N, Maraj B, Bailey A, Carr IM, Andersen S, Cross J, Whelan P, MacLennan KA, Meredith DM, Markham AF: Prostate-specific membrane antigen: evidence for the existence of a second related human gene. Br J Cancer 72: 583–8, 1995
6. O'Keefe DS, Su SL, Bacich DJ, Horiguchi Y, Luo Y, Powell CT, Zandvliet D, Russell PJ, Molloy PL, Nowak NJ, Shows TB, Mullins C, Vonder Haar RA, Fair WR, Heston WD: Mapping, genomic organization and promoter analysis of the human prostate-specific membrane antigen gene. Biochim Biophys Acta 1443: 113–27, 1998
7. Pinto JT, Suffoletto BP, Berzin TM, Qiao CH, Lin S, Tong WP, May F, Mukherjee B, Heston WD: Prostate-specific membrane antigen: a novel folate hydrolase in human prostatic carcinoma cells. Clin Cancer Res 2: 1445–51, 1996
8. Carter RE, Feldman AR, Coyle JT: Prostate-specific membrane antigen is a hydrolase with substrate and pharmacologic characteristics of a neuropeptidase. Proc Natl Acad Sci USA 93: 749–53, 1996
9. Luthi-Carter R, Barczak AK, Speno H, Coyle JT: Molecular characterization of human brain N-acetylated alpha-linked acidic dipeptidase (NAALADase). J Pharmacol Exp Ther 286: 1020–5, 1998
10. Barrett AJ: Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (NC-IUBMB). Enzyme Nomenclature. Recommendations 1992. Suppl 4: corrections and additions (1997). Eur J Biochem 250: 1–6, 1997
11. Troyer JK, Feng Q, Beckett WGL: Biochemical characterization and mapping of the 7E11-CS.3 epitope of the prostate-specific membrane antigen. Urol Oncol 1: 29–37, 1995
12. Liu H, Moy P, Kim S, Xia Y, Rajasekaran A, Navarro V, Knudsen B, Bander NH: Monoclonal antibodies to the extracellular domain of prostate-specific membrane antigen also react with tumor vascular endothelium. Cancer Res 57: 3629–34, 1997
13. Grauer LS, Lawler KD, Marignac JL, Kumar A, Goel AS, Wolfert RL: Identification, purification, and subcellular localization of prostate-specific membrane antigen PSM' protein in the LNCaP prostatic carcinoma cell line. Cancer Res 58: 4787–9, 1998
14. Murphy GP, Greene TG, Tino WT, Boynton AL, Holmes EH: Isolation and characterization of monoclonal antibodies specific for the extracellular domain of prostate specific membrane antigen. J Urol 160: 2396–401, 1998
15. Wright GL, Haley C, Beckett ML, Schellhammer PF: Expression of prostate-specific membrane antigen in normal, benign, and malignant prostate tissue. Urol Oncol 1: 18–28, 1995
16. Bostwick DG, Pacelli A, Blute M, Roche P, Murphy GP: Prostate specific membrane antigen expression in prostatic intraepithelial neoplasia and adenocarcinoma: a study of 184 cases. Cancer 82: 2256–61, 1998
17. Sweat SD, Pacelli, A, Murphy GP, Bostwick DG: Prostate-specific membrane antigen expression is greatest in prostate adenocarcinoma and lymph node metastases. Urology 52: 637–40, 1998
18. Wright GL, Grob BM, Haley C, Grossman K, Newhall K, Petrylak D, Troyer J, Konchuba A, Schellhammer PF, Moriarty R: Upregulation of prostate-specific membrane antigen after androgen-deprivation therapy. Urology 48: 326–34, 1996
19. Kawakami M, Nakayama J: Enhanced expression of prostate-specific membrane antigen gene in prostate cancer as revealed by *in situ* hybridization. Cancer Res 57: 2321–4, 1997
20. Chang SS, Reuter VE, Heston WD, Hutchinson B, Grauer LS, Gaudin PB: Short term neoadjuvant androgen deprivation therapy does not affect prostate specific membrane antigen expression in prostate tissues. Cancer 88: 407–15, 2000
21. Troyer JK, Beckett ML, Wright GL Jr: Detection and characterization of the prostate-specific membrane antigen (PSMA) in tissue extracts and body fluids. Int J Cancer 62: 552–8, 1995
22. Chang SS, Reuter VE, Heston WD, Bander NH, Grauer LS, Gaudin PB: Five different anti-prostate-specific membrane antigen (PSMA) antibodies confirm PSMA expression in tumor-associated neovasculature. Cancer Res 59: 3192–8, 1999
23. Chang SS, O'Keefe DS, Bacich DJ, Reuter VE, Heston WDW, Gaudin PB: Prostate-specific membrane antigen

(PSMA) is produced in tumor-associated neovasculature. Clin Cancer Res, 1999 (in press)
24. Silver DA, Pellicer I, Fair WR, Heston WD, Cordon-Cardo C: Prostate-specific membrane antigen expression in normal and malignant human tissues. Clin Cancer Res 3: 81–5, 1997
25. Elgamal AA, Troychak MJ, Murphy GP: ProstaScint scan may enhance identification of prostate cancer recurrences after prostatectomy, radiation, or hormone therapy: analysis of 136 scans of 100 patients. Prostate 37: 261–9, 1998
26. Petronis JD, Regan F, Lin K: Indium-111 capromab pendetide (ProstaScint) imaging to detect recurrent and metastatic prostate cancer. Clin Nucl Med 23: 672–7, 1998
27. Kahn D, Williams RD, Manyak MJ, Haseman MK, Seldin DW, Libertino JA, Maguire RT: 111Indium-capromab pendetide in the evaluation of patients with residual or recurrent prostate cancer after radical prostatectomy. The ProstaScint Study Group. J Urol 159: 2041–6, 1998
28. Kahn D, Williams RD, Seldin DW, Libertino JA, Hirschhorn M, Dreicer R, Weiner GJ, Bushnell D, Gulfo J: Radioimmunoscintigraphy with 111indium labeled CYT-356 for the detection of occult prostate cancer recurrence. J Urol 152: 1490–5, 1994
29. Michaels EK, Blend M, Quintana JC: 111Indium-capromab pendetide unexpectedly localizes to renal cell carcinoma. J Urol 161: 597-8, 1999
30. Israeli RS, Miller WH, Su SL, Powell CT, Fair WR, Samadi DS, Huryk RF, DeBlasio A, Edwards ET, Wise GJ, Heston WDW: Sensitive nested reverse transcription polymerase chain reaction detection of circulating prostatic tumor cells: comparison of prostate-specific membrane antigen and prostate-specific antigen-based assays. Cancer Res 54: 6306–10, 1994
31. Zhang Y, Zippe CD, Van Lente F, Klein EA, Gupta MK: Combined nested reverse transcription-PCR assay for prostate-specific antigen and prostate-specific membrane antigen in detecting circulating prostatic cells. Clin Cancer Res 3:1215–20, 1997
32. Ghossein RA, Osman I, Bhattacharya S, Ferrara J, Fazzari M, Cordon-Cardo C, Scher HI: Detection of prostatic specific membrane antigen messenger RNA using immunobead reverse transcriptase polymerase chain reaction. Diagn Mol Pathol 8: 59–65, 1999
33. Liu H, Rajasekaran AK, Moy P, Xia Y, Kim S, Navarro V, Rahmati R, Bander NH: Constitutive and antibody-induced internalization of prostate-specific membrane antigen. Cancer Res 58: 4055–60, 1998
34. Eshhar Z, Bach N, Fitzer-Attas CJ, Gross G, Lustgarten J, Waks T, Schindler DG: The T-body approach: potential for cancer immunotherapy. Springer Semin Immunopathol 18: 199–209, 1996
35. Gong MC, Latouche JB, Krause A, Heston DWD, Bander NH, Sadelain M: Cancer patient T cells genetically targeted to prostate-specific membrane antigen specifically lyse prostate cancer cells and release cytokines in response to prostate-specific membrane antigen. Neoplasia 1: 123–127, 1999

Address for correspondence: Michael C. Gong, Department of Urology, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021, USA; *Tel:* 212 639 2273; *Fax:* 917 432 2340, 212 717 3175; E-mail:gongm@mskcc.org